Quarterly Progress Report

Covering Period: October 1 - December 31, 1964

Study of the Normal Fecal Bacterial Flora of Man

RAC 931-6

Prepared Under Contract NASw-738

by

Dr. Lorraine S. Gall

Republic Aviation Corporation
Space Environment and Life Sciences Laboratory
Farmingdale, L. I., New York

Republic Aviation Corporation
Farmingdale, Long Island, New York
SPACE ENVIRONMENT & LIFE SCIENCES LABORATORY

January 7, 1965

Quarterly Progress Report (OCTOBER, NOVEMBER, DECEMBER, 1964)
Contract NASw-738
Study of the Normal Fecal Bacterial Flora of Man

INTRODUCTION

The work conducted this quarter on the predominating normal fecal flora of man centers around two important phases of the study, the primary culturing and isolation of the predominating fecal flora in ten new subjects and further physiological studies on the sixteen type cultures. One more type culture was characterized and was labled FA-18. Some aerobic work proved to be interesting and will be commented upon briefly.

TYPE CULTURES

The procedure used to "establish" type cultures was described in the previous quarterly report number RAC 931-2. Using these methods a new type culture was found and was designated FA-18 (Table 1). This culture is quite distinguishable by its growth characteristic, as growth is manifested largely by production of slime with little or no turbidity occurring. This culture was isolated a total of sixteen times on the new group of ten subjects and was found in other human subjects on another contract being conducted in connection with the establishment of biomedical criteria for personal hygiene at Wright-Patterson Air Force Base. The records pertaining to the first ten subjects have not been checked to see of this organism occurred in those subjects, but this will be done before the next quarterly report.

ISOLATION OF PREDOMINATING FECAL BACTERIA

During this quarter substantial progress was made in the isolation and screening of the predominating fecal anaerobes in ten new subjects. Although this work is still incomplete, report will be made on the results obtained to date. On some subjects all six samples have been obtained whereas the results are in varying state of completion on the other subjects. The preliminary results from this series of subjects compares reasonably well with those obtained on the first ten subjects. Table 2 shows the aerobic plate count in millions from each of the subjects in each time period in which they were sampled and the numbers obtained correspond reasonably well with those

obtained from the first study. It is interesting to note that most of the subjects have a characteristically high or low count with a striking variation occurring only occasionally as in the fifth period on subject 19. In Table 3 the height of anaerobic growth exceeds the aerobic count by the usual 1:1000 to 1:10,000 times. Table 4 shows the number of times that strict anaerobes vs facultative anaerobes occurred in the top three dilutions of the fecal material. As in previous studies, the strict anaerobes are present in the vast majority. On the samples so far tested with the second group of subjects, 92% of the cultures isolated were strict anaerobes, which is somewhat lower than the 95%-97% usually experienced; and it will be interesting to see if the remaining samples to be taken on this group of subjects will show the slightly elevated incidence of facultative anaerobes. Tables 5 through 14 show the incidence of occurrence of the type cultures. It will be noted that a new group GD-1 through GD-7 are included, which are type cultures set up on the basis of data obtained during a study of human subjects on a space-type diet at Wright-Patterson Air Force Base*. These organisms were encountered as a substantial portion of the fecal flora of these young men and increased as the length of time on the space-type diet increased. Table 15 is a summary of the distribution of the various type cultures from all of the subjects by sample period. Although this is incomplete at this time it is interesting to note that the results from this ten subjects when compared with the previous ten subjects shows some strong similarities. For example, on the last ten subjects the cultures found most numerously were FA-15, FA-1, and FA-5, while on the first ten subjects the most numerously isolated cultures were FA-15, FA-3 and FA-5. Thus, two out of the three most numerous organisms were the same in both sets of subjects. FA-3 was a common isolate, but not the most common on the last run, but FA-1 was found rarely on the first ten subjects. However, on a group of human male subjects tested prior to this present contract FA-1 was a frequent isolate. The organisms found most infrequently on the last ten subjects was FA-4, FA-7, FA-9, FA-11, and FA-13, which correlates rather well with the previous study where FA-1, FA-4, FA-7, FA-9, and FA-13 were the least frequent isolated organisms. Thus, four out of the six least frequently isolated cultures correlated well between the two groups of subjects. As was indicated before, the facultative anaerobes were somewhat higher among the screened cultures on the second group of subjects than on the first.

In collaborative tests conducted in conjunction with Dr. Henry A. Dymsza, Dr. G. S. Stoewsand, and Dr. J. J. Enright at the Metabolism Section, Nutrition Branch of Food Division, U. S. Army Natick Laboratories and Dr. P. C. Trexler, of Gnotobiotic Foundation, North Wilmington, Massachusetts, using gnotobiotic rats four "type cultures" isolated and studied under NASA contract NASw-738 were fed to germ-free animals. These four "type cultures" FA-1, FA-9, FA-13 and FA-15 were selected because of their predominance, potential interest and diverse characteristics. It was interesting to note that three of the four "type cultures" fed, increased the weight gain of the rats statistically significantly over the germ-free controls and that two of the "type cultures" decreased the abnormal size of the cecum statistically significantly, but most interesting of all, two of the type cultures increased the amount of cholesterol in the blood plasma by statistically significant amounts.

---

*"Determination of Aerobic and Anaerobic Microflora of Human Feces," AMRL-TR-64-107, Biomedical Laboratory, Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio, October 1964.

PHYSIOLOGY OF TYPE CULTURES

The metabolic studies reported during the last quarter centered around carbohydrate fermentation by the FA type cultures. The work reported here is based on the fermentation of nitrogenous compounds which may be available for bacterial metabolism in the human digestive tract. Investigation of this research area is significant because it may give some insight into the following problem areas: 1) a portion of man's dietary amino acids may be consumed, transformed, or destroyed by intestinal bacteria, 2) chemical by-products of bacterial fermentation may accumulate in the intestine and consequently exert physiological effects on the host, 3) aesthetically objectionable odiferous by-products of bacterial metabolism may be formed.

A wide variety of mechanisms for the microbial utilization of proteins, peptides and amino acids are reported in the literature. However, this study was limited to the metabolism of several classes of substrates which could have ecological significance in the digestive tract. The mechanisms for the degradation of these substrates was primarily limited to deaminating and decarboxylating enzymes. The decarboxylation studies were done with selected amino acids which have a significant role in human nutrition. Only amino acids were chosen which could theoretically be transformed into primary amines having physiological effects on man.

We were ultimately interested in gaining insight into the effects of man's intestinal bacteria on his nutrition. Therefore, deamination studies were done using a pancreatic digest of casein as substrate, to simulate a hydrolyzed protein which would exist in the intestine. The degree of deamination was used as an index to characterize the FA type cultures.

Indol and skatol are some of the major by-products of bacterial metabolism which are responsible for the characteristic odor of feces. These compounds complicate waste management under space conditions because of the objectionable odors. The type cultures were therefore tested for their ability to form indol.

Physiological Results

1. Indol Production

The FA type cultures were tested for their ability to produce indol as a by-product of growth. Type cultures were inoculated into Gall's broth and tryptone broth (Difco) with added cysteine and then incubated anaerobically. The distillation procedure of Gore* (1921) and para-dimethyl-amino benzaldehyde reagent was used to test for indol. The results of these tests indicate that only one of the 16 type cultures, FA-3, produced detectable quantities of indol under the conditions of this experiment.

A major metabolic pathway to indol is through the amino acid tryptophane. Both Gall's broth and tryptone broth contain enzymatic hydrolyzates of casein which are relatively high in tryptophane content. Since only one indol-producing culture

*Gore, S. N., 1921, Indian J. Med. Research, 8, 505-507.

was detected, additional tests were run using Gall's broth and tryptone broth supplemented with tryptophane. However, FA-3 remained the only indol-producing culture of the 16 FA and 7 GD types. As expected, control tests with stock cultures of Escherichia coli were positive and Aerobacter aerogenes were negative. These studies indicate that FA-3 has the unique capability of indol accumulation and may partially explain the characteristic odor of broth cultures.

2. Decarboxylation Studies

The enzymatic decarboxylation of alpha amino acids results in the formation of amines and carbon dioxide:

$$RCH^{*}NH_2)\ CO_2H \xrightarrow{\text{decarboxylase}} RCH_2NH_2 + CO_2$$

The FA type cultures were tested for their ability to decarboxylate the following amino acids to the corresponding amines, all of which are vasoconstrictors:

Amino Acid	Amine
lysine	cadaverine
histidine	histamine
tyrosine	tyramine
arginine	agmatine

In order to screen the cultures a modification of the decarboxylase procedure of Falkow* (1958) was used. FA type cultures were inoculated into appropriate media containing cysteine and the appropriate amino acid, and incubated four days anaerobically. Control cultures of Salmonella and Pseudomonas gave the expected pattern of decarboxylation reactions. Although a taxanomic scheme could be derived on the basis of the decarboxylase tests reported in Table 16, it would be premature until the preliminary data are confirmed manometrically.

3. Deamination

Proteins may be hydrolyzed by extracellular and intracellular bacterial hydrolases, and the constituent amino acids may subsequently be fermented with the formation of ammonia. This process is termed deamination. Proteins are also enzymatically hydrolyzed by a variety of human digestive enzymes to peptides and amino acids, and are then absorbed from the intestine. Bacteria which inhabit the lower intestine scavange the unadsorbed amino acid and peptide.

In order to partially simulate some of the intestinal substrates, pancreatic digests of casein were used in laboratory tests. The 16 FA type cultures were screened for the degree of deamination of casein hydrolyzate using the Conway microdiffusion procedure for ammonia.

Table 17 summarizes the results. The data given are corrected for endogenous ammonia and free ammonia from the substrate and reagents. It is obvious that most of the type cultures are capable of deaminating some of the constituent amino acids in the hydrolyzate.

---

*Amer. J. Clin. Path., 29:598, 1958.

AEROBIC BACTERIA

Although the emphasis in this program is on the predominating fecal flora which have proved to be the anaerobes, aerobic studies are also being carried out at the same time. These procedures include the inoculation of plates containing MacConkey's blood and mitis salivarius agar. The colonies from these plates are picked and certain colonies are studied further. The results obtained from the study of the Enterobacteriaceae are included in Table 18 and show that the vast majority of the gram negative rods isolated on these subjects are E. coli, many of which fall into various serotypes. Occasionally other recognizable gram negative bacteria are found including one typable shigella, poly B. (This man had recently been in contact with chimpanzees carrying this same shigella.) There were several gram negative rods that would not fit into any recognizable pattern, but these were scattered, except for the occurrence of one particular organism which gave a peculiar pattern and occurred repeatedly in the second sampling period on subject 12.

The cocci isolated were shown to be 50% enterococci, about 20% Streptococcus mitis, 20% staphylococci of the coagulase negative type and 10% either unkeyable or showing failure to transplant. These results are based on a total of 40 cultures.

Thus, with the exception of the appearance of so many typable coli, the results of these aerobes are not remarkable.

PROJECTED WORK FOR NEXT QUARTER

During the next quarter it is anticipated that the second series of ten subjects will be completed and that five new subjects will be tested for the types of predominating fecal anaerobes present. Studies will be conducted on the sixteen type cultures including production or utilization of certain B vitamins, and work on the following aspects of the physiology of the type cultures is anticipated: lipolytic enzymes, amylases, and more refined studies on carbohydrate metabolism.

PROJECT PERSONNEL

Personnel who have been working on the program are Dr. Lorraine S. Gall, Charles Huhtanen, Norman Richards, Fay Ames and Shirley Dunwoody.

HOURS EXPENDED: (October 1 - December 31, 1964)

Professional: 438

Technical: 320

L. Gall

Lorraine S. Gall, Ph. D.

LSG/bs

TABLE 1

Screen Tests for Predominating Anaerobic Fecal Bacteria

Culture Number	Morphology	Agar Shake	Broth	Glucose	Sucrose	Lactose	Dextrin	Blank	Litmus Milk	Gelatin	pH
FA-18	gram positive long slender rods, irregular staining	fine colonies, very anaerobic	slight with slime	± moderate slime	± moderate slime	± moderate slime	± moderate slime	± moderate slime	delayed ARC	no liquefaction	6.3 to 6.6
				± moderate slime	± moderate slime	± moderate slime	± moderate slime	± moderate slime			

TABLE 2

Aerobic Plate Count in Millions

Subject No.	Sample Number					
	1	2	3	4	5	6
11	130	75	10	37	35	25
12	50	71	90	14		
13	4	8	6	0	1	
14	0					
15	136	missing	200	210	400	190
16	72	0	1			
17	20					
18	700	800				
19	35	1	3	2	600	45
20	1	1	17	0	17	

TABLE 3

Heighth of Anaerobic Growth by Tube*

Subject No.	Sample Number					
	1	2	3	4	5	6
11	8	9	7	8	9	8
12	9	10	10	10		
13	9	8	9	9	9	
14	9					
15	10	9	10	10	9	8
16	10	8	9			
17	8					
18	10	10				
19	9	8	9	10	9	10
20	10	8	9	8	8	

*(Tube 7 = 10^{-10}; 8 = 10^{-11}; etc.)

TABLE 4

Number of Times Strict Anaerobes vs Facultative Anaerobes
Appeared in the Top Three Dilutions of Growth

	Sample Number											
	1		2		3		4		5		6	
Subject No.	A	F	A	F	A	F	A	F	A	F	A	F
11	2	1	3	0	3	0	2	1	3	0	3	0
12	2	1	2	1	3	0	3	0				
13	3	0	3	0	3	0	3	0	3	0		
14	3	0										
15	3	0	3	0	2	1	3	0	2	1	3	0
16	3	0	3	0	3	0						
17	3	0										
18	3	0	0	3								
19	3	0	3	0	3	0	3	0	3	0	3	0
20	3	0	3	0	3	0	3	0	3	0		

A = Strict Anaerobes
F = Facultative Anaerobes

TABLE 5

Distribution of Anaerobes in Fecal Samples
From Human Subject 11

Anaerobes	Sample Number						
	1	2	3	4	5	6	Total
FA-1	1	2	1	1	1		6
FA-2	1						1
FA-3			2				2
FA-4							
FA-5		3			1		4
FA-6							
FA-7							
FA-8							
FA-9							
FA-10							
FA-11							
FA-12							
FA-13							
FA-14							
FA-15	1		1				2
FA-16							
FA-17			1	1			2
FA-18				2			2
GD-1							
GD-2							
GD-3							
GD-4							
GD-5							
GD-6							
GD-7							
Unkeyed	1	1		2			4
TOTAL	4	6	5	6	2	0	23
FN-1							
FN-2							
FN-3							
FN-4							
Lactobacillus							
Enterococci							
Unkeyed							
TOTAL	0	0	0	0	0	0	0

FA & GD = Obligate Anaerobes
FN = Facultative Anaerobes

TABLE 6

Distribution of Anaerobes in Fecal Samples
From Human Subject 12

Anaerobes	Sample Number						
	1	2	3	4	5	6	Total
FA-1	3	3					6
FA-2							
FA-3	2						2
FA-4							
FA-5	2	4					6
FA-6							
FA-7							
FA-8							
FA-9							
FA-10		1					1
FA-11							
FA-12		1					1
FA-13							
FA-14	1						1
FA-15	5	2	4				11
FA-16							
FA-17							
FA-18							
GD-1							
GD-2							
GD-3							
GD-4							
GD-5							
GD-6							
GD-7							
Unkeyed	3		2				5
TOTAL	16	11	6	0	0	0	33
FN-1							
FN-2							
FN-3							
FN-4		1					1
Lactobacillus							
Enterococci	1						1
Unkeyed							
TOTAL	1	1	0	0	0	0	2

FA & GD = Obligate Anaerobes
FN = Facultative Anaerobes

TABLE 7

Distribution of Anaerobes in Fecal Samples
From Human Subject 13

Anaerobes	Sample Number						
	1	2	3	4	5	6	Total
FA-1	1		1				2
FA-2	1	1					2
FA-3							
FA-4							
FA-5		2					2
FA-6	1			1			2
FA-7							
FA-8							
FA-9							
FA-10							
FA-11	1						1
FA-12							
FA-13			1				1
FA-14							
FA-15	2	2	5	1			10
FA-16							
FA-17				1			1
FA-18							
GD-1							
GD-2							
GD-3							
GD-4			1				1
GD-5							
GD-6							
GD-7							
Unkeyed				1			1
TOTAL	6	5	8	4	0	0	23
FN-1							
FN-2							
FN-3							
FN-4							
Lactobacillus			2				2
Enterococci							
Unkeyed		2	1				3
TOTAL	0	2	3	0	0	0	5

FA & GD = Obligate Anaerobes
FN = Facultative Anaerobes

TABLE 8

Distribution of Anaerobes in Fecal Samples
From Human Subject 14

Anaerobes	Sample Number						
	1	2	3	4	5	6	Total
FA-1	2						2
FA-2							
FA-3							
FA-4							
FA-5							
FA-6							
FA-7	1						1
FA-8							
FA-9	1						1
FA-10							
FA-11							
FA-12							
FA-13							
FA-14							
FA-15	4						4
FA-16	1						1
FA-17							
FA-18	5						5
GD-1							
GD-2							
GD-3							
GD-4							
GD-5							
GD-6							
GD-7							
Unkeyed	2						2
TOTAL	16	0	0	0	0	0	16
FN-1							
FN-2							
FN-3							
FN-4							
Lactobacillus	1						1
Enterococci							
Unkeyed							
TOTAL	1	0	0	0	0	0	1

FA & GD = Obligate Anaerobes
FN = Facultative Anaerobes

TABLE 9

Distribution of Anaerobes in Fecal Samples
From Human Subject 15

Anaerobes	Sample Number						
	1	2	3	4	5	6	Total
FA-1		7	4	1	1		13
FA-2		1		2			3
FA-3					1		1
FA-4				1			1
FA-5		3	5	3			11
FA-6	1				1		2
FA-7							
FA-8							
FA-9							
FA-10							
FA-11							
FA-12							
FA-13							
FA-14							
FA-15	1	1	2	2			6
FA-16	1			1			2
FA-17							
FA-18					1		1
GD-1							
GD-2							
GD-3							
GD-4							
GD-5							
GD-6							
GD-7							
Unkeyed	1		2	1			4
TOTAL	4	12	13	11	4	0	44
FN-1							
FN-2							
FN-3							
FN-4							
Lactobacillus		1					1
Enterococci							
Unkeyed							
TOTAL	0	1	0	0	0	0	1

FA & GD = Obligate Anaerobes
FN = Facultative Anaerobes

TABLE 10

Distribution of Anaerobes in Fecal Samples
From Human Subject 16

Anaerobes	Sample Number						
	1	2	3	4	5	6	Total
FA-1		2	3				5
FA-2							
FA-3		1					1
FA-4							
FA-5			3				3
FA-6							
FA-7							
FA-8	3						3
FA-9							
FA-10		3					3
FA-11							
FA-12	3						3
FA-13							
FA-14							
FA-15		2					2
FA-16							
FA-17							
FA-18		4					4
GD-1							
GD-2							
GD-3		1	1				2
GD-4							
GD-5							
GD-6		1					1
GD-7							
Unkeyed	1	1					2
TOTAL	7	15	7	0	0	0	29
FN-1							
FN-2							
FN-3							
FN-4							
Lactobacillus							
Enterococci							
Unkeyed							
TOTAL	0	0	0	0	0	0	0

FA & GD = Obligate Anaerobes
FN = Facultative Anaerobes

TABLE 11

Distribution of Anaerobes in Fecal Samples
From Human Subject 17

Anaerobes	Sample Number						
	1	2	3	4	5	6	Total
FA-1 FA-2 FA-3 FA-4 FA-5	1						1
FA-6 FA-7 FA-8 FA-9 FA-10	2						2
FA-11 FA-12 FA-13 FA-14 FA-15	 1 4						 1 4
FA-16 FA-17 FA-18 GD-1 GD-2	 1						 1
GD-3 GD-4 GD-5 GD-6 GD-7 Unkeyed	 1						 1
TOTAL	10	0	0	0	0	0	10
FN-1 FN-2 FN-3 FN-4 Lactobacillus Enterococci Unkeyed							
TOTAL	0	0	0	0	0	0	0

FA & GD = Obligate Anaerobes
FN = Facultative Anaerobes

TABLE 12

Distribution of Anaerobes in Fecal Samples
From Human Subject 18

Anaerobes	Sample Number						
	1	2	3	4	5	6	Total
FA-1		6					6
FA-2		1					1
FA-3							
FA-4							
FA-5		2					2
FA-6							
FA-7							
FA-8	4						4
FA-9							
FA-10							
FA-11							
FA-12	1						1
FA-13							
FA-14							
FA-15	2	1					3
FA-16							
FA-17	1						1
FA-18							
GD-1							
GD-2							
GD-3							
GD-4							
GD-5							
GD-6							
GD-7							
Unkeyed	1	3					4
TOTAL	9	13	0	0	0	0	22
FN-1							
FN-2							
FN-3							
FN-4							
Lactobacillus							
Enterococci							
Unkeyed							
TOTAL	0	0	0	0	0	0	0

FA & GD = Obligate Anaerobes
FN = Facultative Anaerobes

TABLE 13

Distribution of Anaerobes in Fecal Samples
From Human Subject 19

Anaerobes	Sample Number						
	1	2	3	4	5	6	Total
FA-1		1	2	1			4
FA-2							
FA-3	1		1				2
FA-4							
FA-5				3			3
FA-6		1					1
FA-7	1						1
FA-8	1						1
FA-9			1				1
FA-10	1		1				2
FA-11	1						1
FA-12							
FA-13		1					1
FA-14			1				1
FA-15	1	2	4	1	1		9
FA-16							
FA-17		1					1
FA-18							
GD-1							
GD-2		1					1
GD-3			4				4
GD-4							
GD-5							
GD-6							
GD-7							
Unkeyed	1	3	1	1			6
TOTAL	7	10	15	6	1	0	39
FN-1							
FN-2							
FN-3							
FN-4							
Lactobacillus			1				1
Enterococci			1				1
Unkeyed							
TOTAL	0	0	2	0	0	0	2

FA & GD = Obligate Anaerobes
FN = Facultative Anaerobes

TABLE 14

Distribution of Anaerobes in Fecal Samples
From Human Subject 20

Anaerobes	Sample Number						
	1	2	3	4	5	6	Total
FA-1	3						3
FA-2							
FA-3							
FA-4							
FA-5		2	2	3			7
FA-6			1	3			4
FA-7							
FA-8					1		1
FA-9							
FA-10			1				1
FA-11							
FA-12							
FA-13							
FA-14							
FA-15							
FA-16							
FA-17					1		1
FA-18	2	1					3
GD-1							
GD-2							
GD-3							
GD-4							
GD-5							
GD-6							
GD-7							
Unkeyed	1		2				3
TOTAL	6	3	6	6	2	0	23
FN-1							
FN-2							
FN-3							
FN-4							
Lactobacillus		1					1
Enterococci							
Unkeyed							
TOTAL	0	1	0	0	0	0	1

FA & GD = Obligate Anaerobes
FN = Facultative Anaerobes

TABLE 15

Summary of Results from All Subjects
by Sample Period

Anaerobes	Sample Number						
	1	2	3	4	5	6	Total
FA-1	10	21	11	3	2		47
FA-2	3	3		2			8
FA-3	3	1	3		1		8
FA-4				1			1
FA-5	2	16	10	9	1		38
FA-6	4	1	1	4	1		11
FA-7	2						2
FA-8	8				1		9
FA-9	1		1				2
FA-10	1	4	2				7
FA-11	2						2
FA-12	4	1					5
FA-13		1	1				2
FA-14	2		1				3
FA-15	20	10	16	4	1		51
FA-16	2			1			3
FA-17	1	1	1	2	1		6
FA-18	8	5		2	1		16
GD-1							
GD-2		1					1
GD-3		1	1	4			6
GD-4			1				1
GD-5							
GD-6		1					1
GD-7							
Unkeyed	12	8	7	5			32
TOTAL	85	75	56	37	9	0	262
FN-1							
FN-2							
FN-3							
FN-4		1					1
Lactobacillus	1	2	3				6
Enterococci	1		1				2
Unkeyed		2	1				3
TOTAL	2	5	5	0	0	0	12

FA & GD = Obligate Anaerobes
FN = Facultative Anaerobes

TABLE 16

Amino Acid Decarboxylase Screening Tests

Culture Designation	Control*	Lysine	Listidine	Tyrosine	Arginine
FA-1	0	0	+	+	+
FA-2	0	0	0	0	+
FA-3	0	+	+	+	+
FA-4	0	0	0	0	0
FA-5	0	0	0	0	0
FA-6	0	0	0	0	0
FA-7	0	0	+	+	+
FA-8	0	0	+	+	0
FA-9	0	+	+	+	+
FA-10	0	+	+	+	+
FA-11	0	0	0	0	0
FA-12	0	+	+	+	+
FA-13	0	+	+	+	+
FA-14	0	+	+	+	+
FA-15	0	0	0	0	+
FA-16	0	0	0	0	+
Pseudomonas sp.	0	0	-	-	0
Salmonella sp.	0	+	-	-	+

* Amino Acid Deficient

Key: + = Decarboxylation, 0 = Negative; - = Not tested

TABLE 17

Ammonia Released From Deamination of Pancreatic Digest of Casein by FA Type Cultures

FA Type	μM Ammonia/Mg. Casein Hydrolyzate
FA-1	>0.15
FA-2	0.02
FA-3	0.07
FA-7	0.14
FA-9	0.18
FA-10	0.15
FA-11	0.23
FA-12	0.32
FA-14	>0.02
FA-15	0.10
FA-16	>0.02

TABLE 18

The Distribution of the Enterobacteriaceae on the Second Group of Ten Subjects

Subject No.	No. Screened	Total Coli	Type Coli	Non-Typable Coli	Other Gram Positive Rods	Miscellaneous
11	5	4	3	1	-	1 not done yet
	3	3	3	0	-	-
	0	-	-	-	-	-
	3	3	0	3	-	-
	4	4	0	4	-	1 not keyed
	3	3	0	3	-	-
12	0	-	-	-	-	-
	10	2	0	2	-	-
	4	1	0	1	Paracolon?(2)	1 unkeyed
13	3	3	3	0	-	-
	0	-	-	-	-	Purification in Process
	3	2	2	0	-	-
	0	-	-	-	-	-
	0	-	-	-	-	-
14	0	-	-	-	-	-
15	0	-	-	-	-	-
	0	-	-	-	-	-
	1	1	0	1	-	-
	4	4	1	3	-	-
	3	1	0	1	-	2 unkeyed
	4	3	2	1	-	1 not done yet

TABLE 18 (cont'd)

The Distribution of the Enterobacteriaceae on the Second Group of Ten Subjects

Subject No.	No. Screened	Total Coli	Type Coli	Non-Typable Coli	Other Gram Positive Rods	Miscellaneous
16	0	-	-	-	-	-
	0	-	-	-	-	-
	0	-	-	-	-	-
17	2	2	2	0	-	-
18	0	-	-	-	-	-
	0	-	-	-	-	-
19	0	-	-	-	-	-
	0	-	-	-	-	-
	2	0	-	-	Hafnia	-
	0	-	-	-	-	-
	4	4	2	2	-	-
	3	3	1	2	-	-
20	0	-	-	-	-	-
	0	-	-	-	-	-
	1	-	-	-	-	Unkeyed
	1	-	-	-	-	-
	0	-	-	-	Shigella Poly B	